Plasmodium knowlesi in travellers, update 2014

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Abstract: OBJECTIVES: Since the initial discovery of Plasmodium knowlesi in Malaysia, cases have been reported from several neighbouring countries. Tourism has also resulted in an increasing number of cases diagnosed in Europe, America, and Oceania. In this review we focus on the risk of the travel-associated acquisition of P. knowlesi malaria. METHODS: A search of the literature in PubMed was carried out to identify articles and literature on the distribution of P. knowlesi infections in Southeast Asia and details of its acquisition and importation by travellers to other continents. The cut-off date for the search was December 1, 2013. Search words used were: "Plasmodium knowlesi", "Plasmodium knowlesi infections", "Plasmodium knowlesi travellers", "Plasmodium knowlesi prevalence", "Plasmodium knowlesi host", "Plasmodium knowlesi vector" "Plasmodium knowlesi RDT", and "Plasmodium knowlesi Malaysia". Traveller numbers to Malaysia were obtained from the Tourism Malaysia website. RESULTS: A total of 103 articles were found. Using a selection of these and others identified from the reference lists of the papers, we based our review on a total of 66 articles. RESULTS: P. knowlesi malaria appears to be the most common malaria species in Malaysian Borneo and is also widely distributed on the Malaysian mainland. Furthermore, locally transmitted cases of P. knowlesi malaria have been reported in Thailand, the Philippines, Vietnam, Singapore, Myanmar, Indonesian Borneo, and Cambodia. Two cases have been reported from non-endemic countries in Asia (Japan and Taiwan) in people with a history of travel to Malaysia and the Philippines. Twelve cases were imported to their home countries by travellers from other continents: two from the USA, two from the Netherlands, two from Germany, and one each from Spain, France, Sweden, Finland, Australia, and New Zealand. In most cases, the infection was associated with a trip to or near forested areas. The symptoms were fever (n=12), headache (n=6), chills (n=6), nausea (n=4), myalgia (n=3), back pain (n=3), abdominal problems (n=1), anorexia (n=2), fatigue (n=2), malaise (n=1), arthralgia (n=1), sore throat (n=1) vomiting (n=2), and jaundice (n=1). All patients were treated successfully with currently available antimalaria treatments. The identification of the pathogen by microscopy can be problematic due to the morphological similarity of P. knowlesi to Plasmodium malariae. CONCLUSION: P. knowlesi appears to be a threat not only to the local population in Malaysia, but also to the estimated 25 million annual tourists and occupational travellers to Malaysia, especially those who visit rural, forested areas of the country. The P. knowlesi risk is not limited to Malaysia, and travellers from Southeast Asia presenting with possible malaria should be considered for a diagnostic work-up that includes P. knowlesi.

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Review

Plasmodium knowlesi in travellers, update 2014

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SUMMARY

Objectives: Since the initial discovery of *Plasmodium knowlesi* in Malaysia, cases have been reported from several neighbouring countries. Tourism has also resulted in an increasing number of cases diagnosed in Europe, America, and Oceania. In this review we focus on the risk of the travel-associated acquisition of *P. knowlesi* malaria.

Methods: A search of the literature in PubMed was carried out to identify articles and literature on the distribution of *P. knowlesi* infections in Southeast Asia and details of its acquisition and importation by travellers to other continents. The cut-off date for the search was December 1, 2013. Search words used were: "*Plasmodium knowlesi*", "*Plasmodium knowlesi* infections", "*Plasmodium knowlesi* travellers", "*Plasmodium knowlesi* prevalence", "*Plasmodium knowlesi* host", "*Plasmodium knowlesi* vector" "*Plasmodium knowlesi* RDT", and "*Plasmodium knowlesi* Malaysia".

Traveller numbers to Malaysia were obtained from the Tourism Malaysia website.

Results: A total of 103 articles were found. Using a selection of these and others identified from the reference lists of the papers, we based our review on a total of 66 articles.

Results: *P. knowlesi* malaria appears to be the most common malaria species in Malaysian Borneo and is also widely distributed on the Malaysian mainland. Furthermore, locally transmitted cases of *P. knowlesi* malaria have been reported in Thailand, the Philippines, Vietnam, Singapore, Myanmar, Indonesian Borneo, and Cambodia. Two cases have been reported from non-endemic countries in Asia (Japan and Taiwan) in people with a history of travel to Malaysia and the Philippines. Twelve cases were imported to their home countries by travellers from other continents: two from the USA, two from the Netherlands, two from Germany, and one each from Spain, France, Sweden, Finland, Australia, and New Zealand. In most cases, the infection was associated with a trip to or near forested areas. The symptoms were fever ($n = 12$), headache ($n = 6$), chills ($n = 6$), nausea ($n = 4$), myalgia ($n = 3$), back pain ($n = 3$), abdominal problems ($n = 1$), anorexia ($n = 2$), fatigue ($n = 2$), malaise ($n = 1$), arthralgia ($n = 1$), sore throat ($n = 1$) vomiting ($n = 2$), and jaundice ($n = 1$). All patients were treated successfully with currently available antimalaria treatments. The identification of the pathogen by microscopy can be problematic due to the morphological similarity of *P. knowlesi* to *Plasmodium malariae*.

Conclusion: *P. knowlesi* appears to be a threat not only to the local population in Malaysia, but also to the estimated 25 million annual tourists and occupational travellers to Malaysia, especially those who visit rural, forested areas of the country. The *P. knowlesi* risk is not limited to Malaysia, and travellers from Southeast Asia presenting with possible malaria should be considered for a diagnostic work-up that includes *P. knowlesi*.

1. Introduction

Plasmodium knowlesi was probably discovered by Franchini in 1927 in its natural simian host, *Macaca fascicularis*, and was described for the first time in morphological detail in 1932 by Knowles and Das Gupta, who also managed to show transmission from monkeys to human volunteers under laboratory conditions.[1–3] Up to 1965, *P. knowlesi* was known only as a simian parasite. This changed when it was found in the blood of a US Army Map Service surveyor who had spent 4 weeks in Malaysia.[4] With a focus on naturally acquired *P. knowlesi* infections in human beings, Singh et al. showed that, in Kapit Division (Sarawak, Malaysia), 58% of 208 microscopically diagnosed malaria patients were actually positive for *P. knowlesi* by PCR. These findings led to the recognition of *P. knowlesi* as the fifth human malaria parasite.[5] Since then different PCR studies have shown that *P. knowlesi* is a common malaria *Plasmodium* species in Malaysian Borneo.[6–9] In 2008, 27.2% of 980 malaria patients from 12 hospitals of Sarawak showed the simian

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malaria infection.[7,8] In Kudat District of the Sarawak neighbour-state Sabah, some 78% of 220 malaria-positive patients were considered to be afflicted with *P. knowlesi* mono-infections and 9% with mixed infections,[8] while in Sandakan Division, 23.6% were reported to be positive for this *Plasmodium* species.[10] Another study in which nested PCR was used in Sabah, showed 63 *P. knowlesi* single infections and two mixed infections out of 107 *Plasmodium spp*-positive samples.[9]

The analysis of archival blood films suggests that, already in 1996, *P. knowlesi* accounted for a large part of microscopically diagnosed *Plasmodium malariae* infections in Sarawak,[11] and according to the more recent investigations, it appears to have increased in recent decades.[12] Also, this *Plasmodium* species has been detected in different states of Peninsular Malaysia.[13,14] However the fifth malaria parasite is not geographically limited to Malaysian Borneo and the Malaysian mainland. In Thailand, the existence of *P. knowlesi* has been reported at different locations throughout the country.[15–17] In Vietnam, three cases have been reported from Ninth Thuan Province[18] and 32 PCR-positive blood samples from Nga Hai and Da Tai (Khanh Hoa Province).[19] Six more cases have been reported in the forested part of Singapore,[20,21] 32 from southern Myanmar,[22] six from the Philippine island of Palawan,[23] and two cases have been described in Cambodia near the border with Thailand.[24]

P. knowlesi is transmitted by mosquitoes of the *Anopheles leucosphyrus* group.[13,25,26] The natural hosts include long-tailed macaques (*M. fascicularis*), pig-tailed macaques (*Macaca nemestrina*), and banded leaf monkeys (*Presbytis melalophos*).[27] The prevalence of infection in long- and pig-tailed macaques in Kapit Division (Sarawak, Malaysia) is up to 78%.[28]

For intercontinental travellers, the significance of *P. knowlesi* infections became clear when, in 2006, a Swedish traveller presented to a Stockholm hospital with a *P. knowlesi*-positive blood picture after a 2-week holiday in Sarawak, Malaysian Borneo.[29] Since then, there have been several reports of imported *P. knowlesi* cases all over the world.[30–39]

This review presents an overview of available knowledge on *Plasmodium knowlesi* with a focus on the risk of travel-associated acquisition of this malaria species. (Figs. 1 and 2)

2. Methods

The data were compiled from searches of PubMed using the following search terms, alone or in combination: "*Plasmodium knowlesi*", "*Plasmodium knowlesi* infections", "*Plasmodium knowlesi* travellers", "*Plasmodium knowlesi* prevalence", "*Plasmodium knowlesi* host", "*Plasmodium knowlesi* vector", "*Plasmodium knowlesi* RDT", and "*Plasmodium knowlesi* Malaysia". References of the selected articles were also searched to complete the documentation. Traveller numbers to Malaysia were sourced from the website of Tourism Malaysia. The cut-off date for the collation of information was December 1, 2013.

3. Results and discussion

We used a selection of the 103 papers resulting from our PubMed screening, with the addition of further papers identified from the references lists. Furthermore we used statistics on tourism in Malaysia obtained from the official website of Tourism Malaysia.

3.1. P. knowlesi *in international travellers (Table 1)*

The first reported case of a naturally transmitted *P. knowlesi* infection concerned a surveyor from the US Army in 1965.[4] This early report suggested that the parasite could also be a threat to international occupational and holiday travellers. Since then, further cases of *P. knowlesi* infection probably acquired during a holiday or work-related trip have been reported from non-endemic countries in Asia ($n = 2$),[40,41] as well as from other continents ($n = 11$).[29–38] In Europe, the two first confirmed imported *P. knowlesi* cases occurred in 2006/2007. The first was a Swedish traveller who had returned from a trekking tour of the jungle area of the Bario Highlands in Malaysian Borneo.[29] The second was a Finnish man who had travelled in Peninsular Malaysia during March 2007.[30] Since then, six more cases have been reported in Europe, in tourists from Spain,[32] France,[36] the Netherlands,[37] and Germany,[38,39] and from a Malay (Sarawak) immigrant to the Netherlands.[33] In the southern hemisphere, two imported cases are known, one in Australia[34] and one in New Zealand.[35] One patient had worked several times in Kalimantan, Indonesian Borneo[34] and the other in Sabah and Sarawak (Malaysian Borneo).[35] A further *P. knowlesi* infection, besides the early 1965 case, was found in the USA, in a female patient who had visited relatives on Palawan Island in the Philippines.[31] From these 12 reported cases in intercontinental travellers, only three cases involved female patients.[31,37,39] The period of travel in the risk area varied from 1 week to several months.[4,29–39] In nearly all cases, a history of travel to a forested area with overnight stays was included.[4,29,30,32–35,37,38] No stay in a forest was reported for only two cases, a female patient visiting relatives on Palawan Island in the Philippines[31] and a French tourist spending his holidays on a tourist beach on the Island of Ko Pyang in Thailand[36]. In both cases, however, the travellers were on the edge of a forested area, but never entered it.[31,36]

3.2. Clinical and laboratory parameters

In a clinical study, Daneshvar et al. reported fever and chills to be the common symptoms, identified in 100% of the investigated cases.[6] Furthermore headache, rigors, malaise, anorexia, myalgia, arthralgia, cough, and abdominal pain were frequently observed. Tachycardia and tachypnea were noted as common clinical signs.[6,42]

In the 12 intercontinental traveller cases, the first common symptom was fever. Other symptoms were headache ($n = 6$), chills ($n = 6$), nausea ($n = 4$), myalgia ($n = 3$), back pain ($n = 3$), abdominal problems ($n = 1$), anorexia ($n = 2$), fatigue ($n = 2$), malaise ($n = 1$), arthralgia ($n = 1$), sore throat ($n = 2$) vomiting ($n = 1$), and jaundice ($n = 1$).[4,29–39] In one case, symptoms of hypoglycaemia with slight hearing and visual loss were reported during treatment with quinine and doxycycline.[30] In most cases, the first clinical symptoms were present 2–13 days after leaving the country of probable acquisition. Only in the case of a 32-year-old Dutch woman were the symptoms already present in the country travelled to.[37] Clinical examination showed an increased temperature (range 38.8–40.2 °C) in all 12 traveller patients.[4,29–38] In only three of 10 cases in whom it was measured, was anaemia found to be present.[29,33,38] Leukocyte counts were lowered in five cases.[29,30,32,34,37] Thrombocytopenia was present in all patients in whom platelet levels were determined.[29–39] Six publications reported an increase in the liver enzyme alanine aminotransferase and five an increased aspartate aminotransferase.[29,32,33,36,37] Renal failure was diagnosed with an increase in creatinine up to 3.45 mg/dl in a German woman.[39]

According to the adapted World Health Organization (WHO) criteria for severe malaria infection by Barber et al., two of the traveller cases could be considered to have had severe infections. In one case this was due to high parasite and bilirubin levels,[33] and in another to rising creatinine levels during hospitalization.[39,42] Concerning the prognosis, Willmann et al. postulated that patients with *P. knowlesi* infections and a parasite count $\geq 35\,000/\mu l$ or $\geq 1\%$, or a platelet count $\leq 45 \times 10^9/l$, should be considered as at risk of

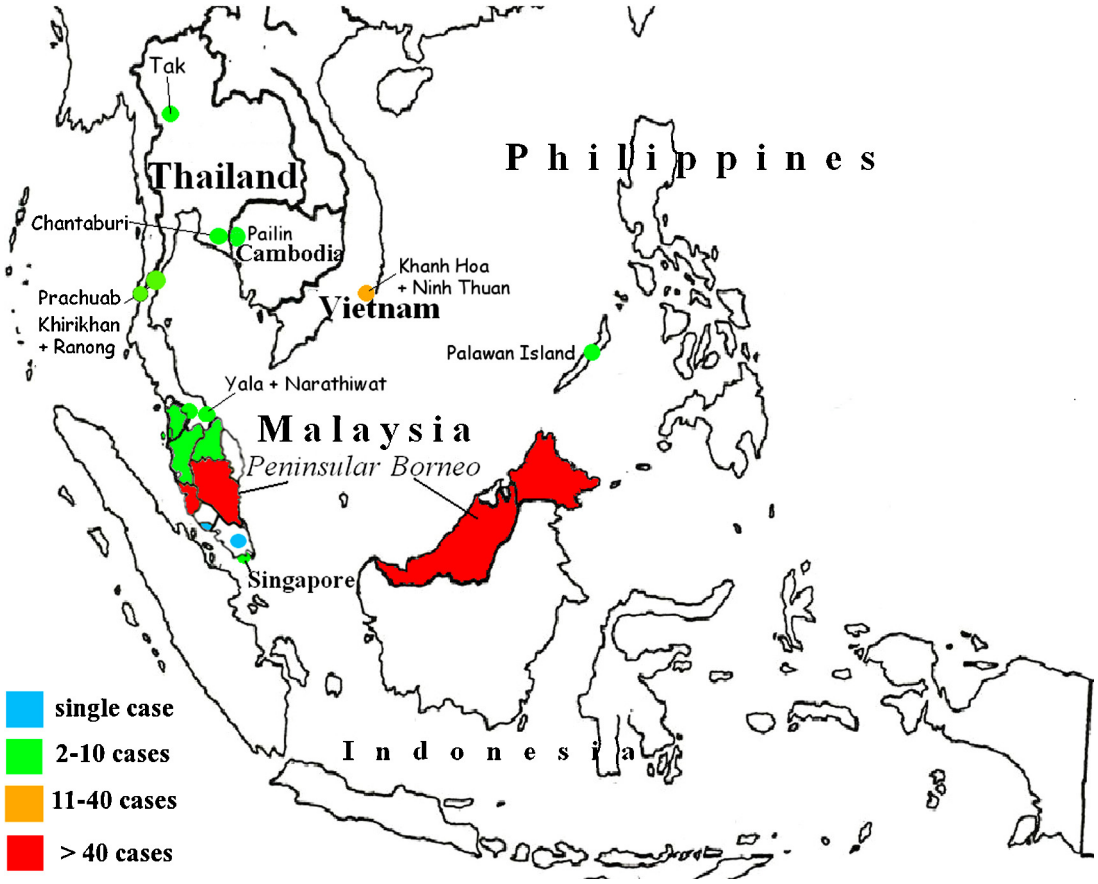

Figure 1. Distribution of reported *Plasmodium knowlesi* infections in Southeast Asia.

further complications.[43] On the other hand Barber et al. reported a correlation only with parasitemia (≥20 000/μl) as a significant marker of severity.[42] Therefore, according to Willmann et al., two more patients could be considered to have been at potential risk of further complications because of their low platelet levels or high parasitemia,[29,31,33,43] and a further case also by the criteria of Barber et al.[4,42] (Table 1 and Table 2)

3.3. *Diagnosis of* P. knowlesi *malaria*

The 'gold standard' for the diagnosis of most *Plasmodium* species is microscopic examination because it is rapid and inexpensive and accurate in expert hands. The disadvantage in its application for the diagnosis of *P. knowlesi* is the large number of false-negatives or misdiagnosed cases due to the morphological similarity with *P. falciparum* in the early trophozoite stage and with *P. malariae* in the late and mature trophozoites, gametocytes, and schizonts.[5,44]

The first nested PCR applied to *P. knowlesi* diagnosis was described by Singh et al.[5] However, in its application, the primer pair resulted in a small number of false-positive results due to cross-reactivity with *Plasmodium vivax*.[45] Since then, additional nested PCR primers and real-time PCR procedures with a higher sensitivity have been developed.[45–48] Although PCR is one of the

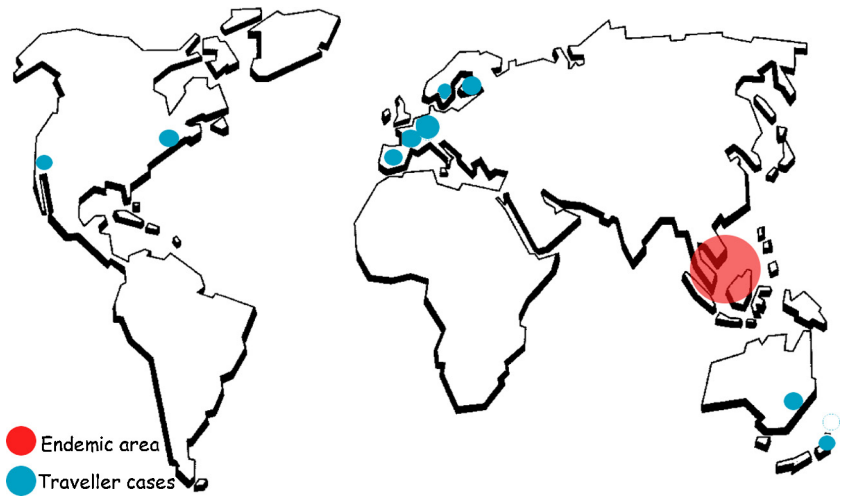

Figure 2. *Plasmodium knowlesi* cases exported from Southeast Asia to other continents.

Table 1
Intercontinental travellers diagnosed with *Plasmodium knowlesi*

Ref.	Year of acquisition	Country of presentation	Presumed area of acquisition	Journey to potential risky areas (forest, jungle)	Interval between leaving the country of acquisition and presentation with symptoms	Symptoms	Treatment	Prophylaxis
Chin et al.[4]	1965	USA	Peninsular Malaysia	NA	NA	Sore throat, chills, fever, sweating, anorexia, fatigue, nausea	Chloroquine, primaquine	NA
Bronner et al.[29]	2006	Sweden	Sarawak, Malaysia, Borneo	Jungle of the Bario Highlands	11 days	Fever (40 °C), sweating, headache, fatigue	Mefloquine	No
Kantele et al.[30]	2007	Finland	Kuala Lumpur, Malaysia; Ipoh, Malaysia; Langkawi, Malaysia	Surrounding areas, jungle	3 days	Fever (38.8 °C), abdominal problems, attack of hypoglycaemia with transient mild hearing and visual loss	Quinine dihydrochloride, doxycycline Later: primaquine	No
CDC[31]	2008	USA	Island of Palawan, Philippines	No (edge of forest area)	NA	Headache, fever, chills	Atovaquone/ proguanil, primaquine	No
Ta et al.[32]	2008/2009	Spain	Bangkok, Thailand Banda Ace + Pulau Weh, Indonesia Kuala Lumpur, Malaysia Hanoi, Vietnam	Rural areas	NA	Fever (40.0 °C), arthralgia, myalgia, low back pain, chills, malaise	Chloroquine	Mefloquine (changed because of adverse drug reaction) Atovaquone/ proguanil (took 80% of prescription)
van Hellmond et al.[33]	2009	Netherlands	Kapit, Sarawak, Malaysia	Jungle	2 days	Fever (40.0 °C), myalgia, headache, low back pain	Chloroquine	-
Figtree et al.[34]	2010[a]	Australia	Kalimanta, Indonesia	Forest	13 days	Fever, mild headaches	Atovaquone/ proguanil	-
Hoosen and Shaw[35]	2010	New Zealand	Sabah, Malaysia; Sarawak, Malaysia	Forest	7–9 days	Fever (39.1 °C), myalgia	Atovaquone/ proguanil Later: artemether/ lumefantrine	-
Berry et al.[36]	2010	France	Ranong, Thailand; Island of Ko Payam, Thailand	No (edge of forest area)	9 days	Fever, shivering, nausea, anorexia	Chloroquine	-
Link et al.[37]	2011	Netherlands	Borneo, Malaysia	Jungle	1 day before leaving country of probable acquisition	Fever (40.2 °C), chills, nausea, vomiting, severe, headache, backache	Atovaquone/ proguanil	No
Ehrhardt et al.[38]	2013[a]	Germany	Ranong, Thailand	Forest	9 days	Fever (40.0 °C), chills, severe, headaches	Atovaquone/ proguanil	No
Orth et al.[39]	2013[a]	Germany	Khoa Sok, Thailand	National Park	10 days	Fever, nausea, vomiting	Artesunate, artemether/ lumefantrine	-

NA, information not available.
[a] The year of infection was unclear, so the year of publication has been used.

most sensitive and specific known diagnostic methods, it requires time, money, and well-trained personnel, and for these reasons it is not ideal in most settings.[49]

Currently available rapid diagnostic tests (RDTs) based on monoclonal antibodies (MAbs) capturing pan-Plasmodium lactate dehydrogenase (pan-pLDH) and genus-specific aldolase (pan-aldolase), only have a sensitivity of 74% and 23%, respectively, and do not distinguish between the non-falciparum species.[50] In 2008, the team of McCutchan analysed the reactivity of known Plasmodium LDH-specific MAbs. In this experiment, McCutchan et al. were able to distinguish *P. knowlesi* from *P. malariae* infections. However the detection of *P. knowlesi* infections is confounded by the difficulty in distinguishing *P. knowlesi* from mixed falciparum–vivax infections.[51] In 2011, Piper et al. developed a model for a new test format based on three test strips, with which the four human *Plasmodium* species and *P. knowlesi* could be differentiated.[52]

Iseki et al. in Japan (2010) and Lau et al. in Malaysia (2011) created *P. knowlesi*-specific primers against β-tubulin[53] and apical membrane antigen-1,[49] which can be used in a loop-mediated isothermal amplification (LAMP). The advantages compared to PCR are the velocity (10^9 copies of a targeted gene in 1 h) and the required isotherm conditions, which allow the use of simpler and cheaper techniques such as an incubator.[53] The exact niche for this diagnostic approach has yet to be established.

3.4. Treatment and chemoprophylaxis

The most frequently reported drug treatment for *P. knowlesi* infections in the papers reviewed was a combination of chloroquine and primaquine.[4–6,8,23,54] Due to the fact that no hypnozoites have ever been described in *P. knowlesi* infections, primaquine is probably not required as a treatment, except in cases of mixed infection with *P. vivax*. In one study, the time to clear 90% of the parasitemia present at admission was defined as 10.3 h with a fever remission time of 26.5 h in adults. The dosage used was 25 mg/kg chloroquine over 2 days and additionally 15 mg primaquine.[54] Children with the same drug combination showed a much slower mean parasite clearance of 2 days. A significant correlation has been shown between the parasite concentration in the blood and the time to parasite clearance.[8] A recent study revealed the efficiency of artemisinin-based combination therapy (ACT) with artemether–lumefantrine and artesunate–mefloquine for non-severe *P. knowlesi* infections, or intravenous artesunate for severe infections.[42] A faster parasite clearance was reported with artemether–lumefantrine and artesunate compared to chloroquine and quinine.[42] Even if at the present time, large studies on the other established malaria treatments are missing, several single reports have underlined the efficiency of the different currently available drugs. The documented successful treatment with different combinations of chloroquine, primaquine, artemisinin combinations, sulfadoxine, pyrimethamine, and quinine are shown in Table 5. Mefloquine was described three times as an effective therapy.[24,29,40] In another case report involving a re-infected Malaysian businessman during an expedition in Tanjung Malim, Perak, Malaysia, the parasitemia continued to increase despite a total administration of 1.5 g of mefloquine. The man, who presented symptoms of chills, rigors, epigastric pain, nausea, and vomiting after an expedition with overnight camping, was started on initial therapy of 750 mg mefloquine with two successive doses of 500 mg after 6 h and 250 mg after 12 h. Despite treatment, the parasitemia increased from 1.0% to 2.5%. Medication was changed to quinine, doxycycline, and the combination artemether and lumefantrine. Parasitemia eventually cleared on day 5 of admission to the hospital.[55] There may be potential resistance of some *P. knowlesi* strains against mefloquine. Experiments with infected rhesus monkeys showed a suppressive activity of mefloquine but without complete cure against *P. knowlesi* infections.[56,57] Recent studies have indicated an innate immunity to mefloquine in vitro, as well in ex vivo experiments.[58]

Data regarding chemoprophylaxis for prevention are missing, but the fact that none of the infected cases reported so far had received adequate prophylaxis could indicate an effect of known preventive anti-malaria drugs against *P. knowlesi* as well. Only one case, a Spanish traveller, had used mefloquine and later atovaquone/proguanil chemoprophylaxis; this case developed a mild *P. knowlesi* infection but later recovered without further treatment.[32] (Table 3, Table 4 and Table 5).

3.5. Risk and advice for travellers

In 2012, 25 032 708 tourist arrivals were registered in Malaysia. Most of them were from Asian countries, especially from Singapore, Thailand, Indonesia, Brunei, and China. In the same year, Tourism Malaysia reported an increase to 1 002 067 European travellers (from the UK, France, Germany, Netherlands, Italy, Sweden, Switzerland, Denmark, Spain, Ireland, Norway, Belgium, Austria, and Poland), 327 065 from the USA and Canada, and 573 674 from New Zealand and Australia.[59] The risk of transmission therefore appears low for intercontinental travellers considering that only 14 cases have been reported beyond endemic areas.

Table 2
Laboratory parameters of intercontinental travellers with *Plasmodium knowlesi* infections

Ref.	Case	Temp., °C	Leukocytes[a]	Thrombocytes[a]	ALT[a]	AST[a]	Parasitemia
Chin et al.[4]	USA, 1965	40.4	NA	NA	NA	NA	20 850/μl
Bronner et al.[29]	Sweden, 2006	40.0	2.2×10^9/l ↓	34×10^9/l ↓	1.68 μcat/l ↑	NA	NA
Kantele et al.[30]	Finland, 2007	38.8	2.6×10^9/l ↓	143×10^9/l ↓	NA	NA	<1%
CDC[31]	USA,2008	NA	NA	Thrombocytopenia not specified	NA	NA	2.9%
Ta et al.[32]	Spain, 2008/2009	40.0	3.82×10^9/l ↓	86×10^9/l ↓	93U/l ↑	43 U/l ↑	0.003% 250/μl
van Hellmond et al.[33]	Netherlands, 2009	40.0	5.8×10^9/l	22×10^9/l ↓	199 U/l ↑	128 U/l ↑	2% 84 000/μl
Figtree et al.[34]	Australia, 2010[b]	38.9	3.7×10^9/l ↓	106×10^9/l ↓	NA	NA	185/μl
Hoosen and Shaw[35]	New Zealand, 2010	39.1	4.3×10^9/l	71×10^9/l ↓	NA	NA	NA
Berry et al.[36]	France, 2010	NA	Normal	73×10^9/l ↓	75 U/l ↑	58 U/l ↑	0.8%
Link et al.[37]	Netherlands, 2011	40.2	3.5×10^9/l ↓	72×10^9/l ↓	80 U/l ↑	76 IU/l ↑	0.0005%
Ehrhardt et al.[38]	Germany, 2013[b]	40.0	4.1×10^9/l	197×10^9/l	NA	NA	0.01% 473/μl
Orth et al.[39]	Germany, 2013	NA	7.36×10^9/l	27×10^9/l ↓	277 U/l ↑	237 U/l ↑	0.2%

ALT, alanine aminotransferase; AST, aspartate aminotransferase; NA, information not available.
[a] ↓ Value lower than locally used reference value; ↑ value higher than locally used reference value.
[b] The year of infection was unclear, so the year of publication has been used.

Table 3
Diagnostic methods used to investigate *Plasmodium knowlesi* infections in intercontinental travellers, and the results

Ref.	Case	Microscopy	RDTs	Nested PCR	Real-time PCR	Other or not nearer specified PCRs
Chin et al.[4]	USA, 1965	Positive for *P. falciparum* (1st examination) Positive for *P. malariae* (2nd examination) Later positive for *P. knowlesi*[a]	-	-	-	-
Bronner et al.[29]	Sweden, 2006	Suspicion for *P. malariae*	Negative (BinaxNOW[b])	Positive for *P. knowlesi* (nested PCR and sequencing of the SSU rRNA)	-	Negative (PCR specific for *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*)
Kantele et al.[30]	Finland, 2007	Positive for *P. falciparum* (1st examination) Positive for co-infection of *P. falciparum* and *P. malariae* (2nd examination)	-	Negative (nested PCR with rOva1 and rPLU2 primers) Positive for *P. knowlesi* (nested PCR and sequencing with rPLU6 and rPLU2 primers)	-	-
CDC[31]	USA, 2008	Positive for unspecific malaria	-	-	-	Negative for: *P. malariae*, *P. falciparum*, *P. ovale*, *P. vivax* (conventional PCR targeting the SSU rRNA) Positive for *P. knowlesi* (conventional PCR with primer specific for the SSU rDNA of *Plasmodium* genus and sequencing)
Ta et al.[32]	Spain, 2008/2009	Positive for unspecific malaria	Negative for HRP2 Negative for pan-malarial aldolase (BinaxNOW[b])	-	Positive for *P. knowlesi* (real-time PCR with sequencing of the SSU rRNA)	Positive for *P. knowlesi* (multiplex real-time PCR specific for *Plasmodium* genus, *P. falciparum*, *P. vivax* and sequencing)
van Hellmond et al.[33]	Netherlands, 2009	Positive for unspecific malaria	Negative for HRP2 Positive for pan-malarial aldolase (BinaxNOW[b])	Negative for *P. malariae*, *P. falciparum*, *P. ovale*, *P. vivax* (nested PCR) Positive for *P. knowlesi* (nested PCR with rPLU1 and rPLU5 primers, species-specific primers targeting the SSU rRNA and sequencing)	Negative for *P. malariae*, *P. falciparum*, *P. ovale*, *P. vivax* (real-time PCR)	-
Figtree et al.[34]	Australia, 2010[c]	Positive for *P. malariae* or *P. falciparum*	Negative for HRP2	Positive for *P. knowlesi* (nested PCR with *P. knowlesi*-specific primers (Pmk8 Pmkr9) and sequencing of SSU rRNA)	-	Negative for *P. malariae*, *P. falciparum*, *P. ovale*, *P. vivax* (multiplex PCR)
Hoosen and Shaw[35]	New Zealand, 2010	Negative (1st and 2nd examination) Suspected *P. malariae* (3rd examination)	Negative for *P. falciparum* and other *Plasmodium* antigen	-	-	Positive for *P. knowlesi* (PCR and sequencing)
Berry et al.[36]	France, 2010	Positive for mixed *P. malariae* and *P. falciparum* infection (1st and 2nd examinations)	Positive for *P. vivax*-specific LDH Positive for pan-*Plasmodium* LDH Negative for HRP2 (Core Malaria Pan/Pv/Pf test[d]) Positive for pan-*Plasmodium* aldolase Negative for HRP2 (BinaxNOW[e]) Positive for *P. vivax*-specific LDH Positive for pan-*Plasmodium* LDH Negative for HRP2 (Palutop test[f])	-	Positive for malaria Negative for *P. falciparum* (multiplex real-time PCR) Positive for *P. vivax* Negative for: *P. ovale*, *P. malariae* (real-time PCR specific for *P. malariae*, *P. vivax*, *P. ovale*) Positive for *P. knowlesi* (sequencing)	-

Table 3 (*Continued*)

Ref.	Case	Microscopy	RDTs	Nested PCR	Real-time PCR	Other or not nearer specified PCRs
Link et al.[37]	Netherlands, 2011	Negative (1st examination) Positive for *P. malariae* (2nd examination)	Negative (BinaxNOW[e])	-	Negative (real-time PCR specific for *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*) Positive for *P. knowlesi* (novel real-time PCR specific primers for *P. knowlesi* SSU rRNA)	-
Ehrhardt et al.[38]	Germany, 2013	Positive for mixed *P. malariae* and *P. falciparum* infection	-	-	-	Positive for *P. knowlesi* (PCR and sequencing)
Orth et al.[39]	Germany, 2013	Positive for *P. malariae*	Negative for HRP2 (BinaxNOW[e]) Positive for Pan-*Plasmodium* aldolase (BinaxNOW[e])	-	-	Positive for *P. knowlesi* (PCR and sequencing)

RDT, rapid diagnostic test; SSU, small subunit; LDH, lactate dehydrogenase; HRP2, *P. falciparum*-specific histidine-rich protein 2.
[a] Later positive for *P. knowlesi* examination of blood inoculated in rhesus monkeys and gibbons.
[b] BinaxNOW Malaria Test, Binax Inc., Maine, USA.
[c] The year of infection was unclear, so the year of publication has been used.
[d] Core Malaria Pan/Pv/Pf test, Core Diagnostics, Birmingham, UK.
[e] BinaxNOW Malaria Test, Iverness Medical, Sevres, France.
[f] Palutop Test, All Diag, Strasbourg, France.

Since Malaysian coastal and urban areas have been declared malaria-free zones by the WHO, travellers at potentially high risk are those undertaking trips and trekking tours in forested or rural areas in the deep hinterland. A lower risk of *P. knowlesi* infection is reported from Thailand, but certain forested areas are considered as malaria zones.[60] The recent report of a French man acquiring a *P. knowlesi* infection probably on a tourist beach on the Island of Ko Payam, Thailand, indicates a risk in coastal areas as well, particularly if they are situated near a forest.[36]

People travelling to known risk areas for *P. knowlesi* should use malaria vector protection measures, such as insect repellents,[61] mosquito nets, mosquito coils, aerosol sprays, and protective clothes.[60] Until more specific research contradicts this doctrine, currently available chemoprophylaxis medication is effective against *P. knowlesi*.[54] A single case of possible resistance to mefloquine has been reported in a Malaysian businessman, as mentioned above.[55] Patients presenting in non-endemic countries with malaria-typical symptoms, such as fever, headache, malaise,

Table 4
Infection associated with local travel in Southeast Asia

Ref.	Year of infection	Country of presentation	Travel areas	Journey to potential risky areas (forest, jungle, etc.)	Time to clinical manifestations after leaving the place of potential acquisition	Method of diagnosis	Prophylaxis
Jongwutiwes et al.[15]	2000	Bangkok, Thailand	Prachuap, Khiri Khan Province, Thailand	Forest	1 week	PCR	-
Ng et al.[21] Jeslyn et al.[20]	2007 (5 cases) 2008 (1 case)	Singapore	Lim Chu Kang, Singapore	Forest	1–2 weeks	PCR	-
Cox-Singh et al.[65]	2010[a]	Kota Kinabalu, Sabah, Malaysia	Jungle of Borneo	Jungle	10 days	PCR	-
Lau et al.[55]	2009	Malaysia	Raub, Pahang, Malaysia Tanjung Malim, Perak Malaysia	Jungle	2 weeks	PCR	-
	2010	Malaysia		Jungle	15 days	PCR	-
Lee et al.[66]	2008	Klang Valley, Peninsular Malaysia	Klang Valley, Peninsular Malaysia	Jungle	2–3 weeks	PCR	-
Lee et al., [66]	2007	Klang Valley, Peninsular Malaysia	Bario Sarawak, Malaysia	-	-	PCR	-
Lee et al., [66]	2008	Klang Valley, Peninsular Malaysia	Pahang, Malaysia	Forest	2–3 weeks	PCR	-
Lee et al., [66]	2008	Klang Valley, Peninsular Malaysia	Ampang, Malaysia	Jungle	3–4 weeks	PCR	-
Lee et al., [66]	2008	Klang Valley, Peninsular Malaysia	Labuan, Malaysia	Jungle	1 week	PCR	-
Lee et al., [66]	2008	Klang Valley, Peninsular Malaysia	Kuala Kubu, Selangor, Malaysia	Riverside	1 week	PCR	-
Lee et al., [66]	2008	Klang Valley, Peninsular Malaysia	Klang Valley, Peninsular Malaysia	KKB, Malaysia	-	PCR	-

[a] The year of infection was unclear, so the year of publication has been used.

anorexia, myalgia, cough, nausea, abdominal pain, or vomiting, and a history of travel to Malaysia, Myanmar, Thailand, Vietnam, or the state of Palawan Philippines in the last 2 weeks, should be investigated for a potential *P. knowlesi* infection.[6] (Table 2)

The high rate of misdiagnosis of *P. knowlesi* infection as *P. falciparum* or *P. malariae* by light microscopy in non-endemic countries (Table 3) should lead to a new evaluation of diagnostic techniques.[29–39] PCR shows a high specificity and sensitivity, but due to the complex process and high costs is not ideal.[34,49] LAMP, as described by Iseki et al. and Lau et al., could be a possible faster and cheaper diagnostic tool, but further studies should be done to evaluate the efficiency of this procedure.[49,53] A recent publication showed a low sensitivity of currently available RDTs in detecting *P. knowlesi*. The development of more sensitive tests would also bring the advantage of cheaper and faster handling under ambulatory conditions.[42] The latest research on protein structures as markers for the detection of *P. knowlesi*, indicates promising progress in this field.[62] Until ideal commercial diagnostic tests are developed, it is

Table 5
Drug treatment of *Plasmodium knowlesi*

Ref.	Drug	Count of cases	Dose	Outcome
Singh et al.[5]	Chloroquine, primaquine	92	*n* = 38: Chloroquine 450–1350 mg (over 1–3 days); primaquine 15 or 7.5 mg (over 2–3 days/2 weeks) *n* = 29: Chloroquine 1500, 1200, or 1050 mg (over 3 days); primaquine 15 or 7.5 mg/day (for 3 days) *n* = 14: Chloroquine 1200 or 1500 mg (over 3 days); primaquine 15 or 7.5 mg/day (over 2–3 days) *n* = 11: Chloroquine 1500 mg (over 3 days); primaquine 15 or 7.5 mg (over 2–3 days/2 weeks)	Survived
Daneshvar et al.[6]	Chloroquine, primaquine	97	Chloroquine 25 mg/kg (over 3 days); primaquine 15 mg/day (over 2 days)	Survived
Daneshvar et al.[54]	Chloroquine, primaquine	69	Chloroquine 25 mg/kg (over 48 h); primaquine 15 mg	Survived
Luchavez et al.[23]	Chloroquine, primaquine	2	Chloroquine 1500 mg (over 72 h); primaquine 45 mg (over 24 h)	Survived
Chin et al.[4]	Chloroquine, primaquine	1	-	Survived
Barber et al.[8]	Chloroquine, primaquine (in children)	13	-	Survived
Jongwutiwes et al.[15]	Chloroquine	1	25 mg/kg (over 72 h)	Survived
Ng et al.[21]	Chloroquine	1	900 mg (over the first 6 h); 600 mg/kg (over following 2 days)	Survived
Berry et al.[36]	Chloroquine	1	500 mg (1×/day over 5 days)	Survived
van Hellmond et al.[33]	Chloroquine	1	10 mg/kg; 5 mg/kg	Survived
Lee et al.[66]	Chloroquine	-	-	Survived
Singh et al.[5] Barber et al.[8]	Quinine	5	-	Survived
Daneshvar et al.[6]	Quinine	8	-	Survived
Daneshvar et al.[6]	Quinine	2		Died
Cox-Singh et al.[7]	Quinine	1	-	Died
Bronner et al.[29] Khim et al.[24] Tanizaki et al.[41]	Mefloquine	3	-	Survived
Lau et al.[55]	Mefloquine (resistance) Later: quinine, doxycycline Later: quinine, artemether, lumefantrine	1	Mefloquine 1500 mg - -	Survived
Lau et al.[55] Lee et al.[66]	Quinine Doxycycline	4	-	Survived
Kantele et al.[30]	Quinine, doxycycline Later: primaquine	1	-	Survived
Lee et al.[66]	Quinine, doxycycline Later: chloroquine	1	- Chloroquine 150 mg	Survived
Barber et al.[42]	Artemether, lumefantrine	34	-	Survived
Barber et al.[42]	Artesunate, mefloquine	119	-	Survived
Barber et al.[42]	Artemether, lumefantrine, artesunate (intravenous)	75	-	Survived
Orth et al.[39]	Artesunate (intravenous) Later: artemether, lumefantrine	1	Artesunate 2.4 mg/kg (1 day) Artemether/lumefantrine	Survived
Figtree et al.[34]	Atovaquone, proguanil	1	Atovaquone 250 mg; proguanil 100 mg (4×/day over 3 days)	Survived
Link et al.[37] Ehrhardt et al.[38]	Atovaquone, proguanil	2	Atovaquone 1000 mg/day; proguanil 400 mg/day (over 3 day)	Survived
Hoosen and Shaw[35]	Atovaquone, proguanil Later: artemether, lumefantrine	1	-	Survived
CDC[31]	Atovaquone, proguanil Later: primaquine	1	-	Survived
Singh et al.[5]	Chloroquine, primaquine, sulfadoxine, pyrimethamine	10	Chloroquine + primaquine; sulfadoxine 1000 mg (single dose); pyrimethamine 50 mg (single dose)	Survived
Cox-Singh et al.[7]	Chloroquine, sulfadoxine, pyrimethamine	2	-	Died
Cox-Singh et al.[7]	Chloroquine, sulfadoxine, pyrimethamine, primaquine Later: quinine	1	-	Died

important to use the established diagnostic tools in the most efficient way. This is especially important for infections with morphological structures similar to *P. malariae* on light microscopy in those with a history of travel to Malaysia and a low platelet count, who should be suspected of having a possible *P. knowlesi* infection.[2,8,9] (Table 4)

Immediate hospitalization and initiation of treatment are imperative due to the 24-h replication cycle and the potential severity of the fifth human malaria.[6,7] Indicators for a potential severe course have been described by Willmann et al.[43] and Barber et al.,[42] as mentioned above. Patients showing signs of a severe knowlesi infection should be treated according to the guidelines for severe *P. falciparum* infections, with parenteral medication.[42,60,63]

Several treatment regimens are reported to be effective against *P. knowlesi*, including the chloroquine/primaquine combination.[54] Due to the fact that no hypnozoites have ever been described in *P. knowlesi* infection, primaquine is probably not required as a treatment except in cases of mixed infection with *P. vivax*. Further studies are needed to evaluate the optimal prevention and treatment of *P. knowlesi*, especially in view of increasing resistance to the concomitantly occurring *P. falciparum* in Southeast Asia. Recent studies have shown the efficacy of ACT, such as artemether–lumefantrine and artesunate–mefloquine, in non-severe *P. knowlesi* infections, as well as intravenous artesunate in severe cases.[42,64] These treatment regimens appear promising, particularly in view of the often multi-resistant, co-endemic *P. falciparum* and *P. vivax* infections. In addition, ACTs have a significantly faster parasite clearance compared to chloroquine.[64]

4. Conclusions

P. knowlesi appears to be a widespread human malaria parasite in Malaysian Borneo[65,66]. This paper illustrates that the fifth human parasite is not only a threat to residents, but also to occupational and holiday travellers. Despite the increasing prevalence in Malaysia, infection rates in travellers remain low, but the extent of misdiagnosed cases remains uncertain. A special risk group are travellers to forested and rural areas. At lower, but still underestimated risk of *P. knowlesi* infection, are travellers to Thailand, the Philippines, Indonesia, Myanmar, and Vietnam, and probably also Cambodia. Clinicians who provide pre-travel advice or who consult ill returning travellers should be aware of the risk. Adequate preventive measures, such as mosquito nets, insect repellents, and the wearing of protective clothing, should be recommended together with appropriate antimalarials. In travellers who present with malaria-typical symptoms and a recent history of travel to Malaysia or neighbouring areas in Southeast Asia, a probable *P. knowlesi* infection has to be taken into consideration. If blood smears are positive for *P. malariae*, a *P. knowlesi* infection has to be strongly suspected, especially if accompanied by low thrombocyte counts. Due to the fast reproduction cycle and a potentially severe progression, patients who are suspected of having a *P. knowlesi* infection should be hospitalized and treated immediately.

Conflict of interest: The authors declare that they have no conflict of interest with this work.

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